The Physiological Effects of Multiple Forced Submergence in Loggerhead Sea Turtles (*Caretta caretta*) During Trawling

Final Report to National Marine Fisheries Service, Galveston Laboratory

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Abstract

Sea turtles are subjected to involuntary submergence and potential mortality due to incidental capture by the commercial fishing industry. Despite implementation of turtle excluder devices (TEDs) to reduce at-sea mortality, dead stranded turtles continue to be found in near-record numbers along the coasts of the western Atlantic Ocean and northern Gulf of Mexico. One plausible explanation for this continued mortality is that sea turtles are repetitively submerged in legal TEDs of one vessel following another. A laboratory study conducted in 1997-1998 revealed that multiple submergence of loggerhead sea turtles (*Caretta caretta*) induced a significant acid-base disturbance (Stabenau and Vietti, 1998). Recovery of blood homeostasis in that study was dependent on the interval between the submergence episodes. However, turtles in that study were confined during the submersion episodes, a condition that does not occur during exposure in TED-equipped commercial fishing nets. The present study was designed, therefore, to examine the physiological effects of multiple enforced submergence in loggerheads following release into and escape from TED-equipped shrimp nets. Pre- and post-submergence blood samples were collected from turtles submerged three times at 7.5 min per episode with a rest interval of 10, 42 or 180 min between submergences. No turtles died during the course of this study. Analyses of the pre- and post-submergence blood samples revealed that the initial submergence produced a severe and pronounced metabolic and respiratory acidosis in all turtles. Successive submergences produced significant changes in blood pH, Pco_2, and lactate, although the magnitude of the acid-base imbalance was substantially reduced as the number of submergences increased. In addition, increasing the interval between successive submergences permitted greater recovery of blood

homeostasis. Taken together, these data suggest that repetitive submergence of sea turtles in TEDs would not significantly affect their survival potential provided the animal has an adequate rest interval at the surface between successive submergences.

Introduction

The five sea turtle species inhabiting the waters of the U.S. Gulf of Mexico and Atlantic Ocean are considered to be threatened or endanger of extinction. One contributing factor to sea turtle mortality is incidental capture in the nets of commercial shrimping vessels. The National Research Council's Committee on Sea Turtle Conservation (1990) suggested that as many as 5,500 to 55,000 loggerhead (*Caretta caretta*) and Kemp's ridley (*Lepidochelys kempi*) sea turtles were killed annually during shrimping-related activities. More recently, two independent studies statistically confirmed the relationship between shrimping activity and the appearance of stranded sea turtles in the U.S. Gulf of Mexico and the Atlantic Ocean (Caillouet *et al.*, 1991; Crowder *et al.*, 1995). Due to the impact of trawl-related mortality on sea turtle populations, the U.S. government passed legislation in 1987 requiring that commercial shrimping vessels pull nets equipped with certified turtle excluder devices (TEDs). TEDs are designed to exclude any turtle that may enter into shrimping nets, while not affecting catch of the target species. Crowder *et al.* (1995) reported that the sea turtle population off the coast of South Carolina continued to decline when TED regulations were implemented, although the rate of decline was significantly less since full-time TED use.

In spite of the TED regulations, near-record numbers of dead stranded sea turtles have been found on U.S. Gulf of Mexico and Atlantic Ocean beaches (W. Teas, pers. comm.). While there may be other man-related or natural causes for this continued sea turtle mortality, there are two plausible reasons for the mortality to be caused during shrimping activities. First, commercial shrimp fishermen are not carrying legally certified TEDs, which may occur with improper installation or by purposely sewing them shut.

Second, the shrimp fishermen are pulling legal TEDs; however, the turtles are repetitively involuntarily submerged as they are caught in the TEDs of vessels following each other. This successive submergence may compound the physiological effects experienced by sea turtles during an involuntary submersion, and thus, may limit their survival potential.

Sea turtles spend all but 1% of their time under the surface of the water. During the brief period at the surface, the turtle will exhale and inhale a solitary breath and then dive under the surface (Jackson, 1985). In fact, multiple breaths by sea turtles are seen only after prolonged dives. Scant information is available on the physiological effects of voluntary submergence of sea turtles. It has been suggested that voluntary dives by sea turtles are aerobic in nature (Wood *et al.*, 1984), whereby oxygen availability minimizes the metabolic production of lactic acid. The turtles may accumulate carbon dioxide, resulting in a respiratory acidosis that is ameliorated by hyperventilation at the surface. Obviously, voluntary diving does not limit sea turtle survival potential.

In contrast, involuntary submergence of Kemp's ridley and loggerhead sea turtles produces significant blood respiratory and metabolic derangements. Stabenau *et al.* (1991) reported that involuntary submergence of Kemp's ridley sea turtles for less than 7.5 min in shrimp nets equipped with TEDs resulted in significant increases in blood lactic acid and Pco_2, and decreases in blood pH. Moreover, several hours were required for turtles to fully recover blood homeostasis (E. Stabenau, pers. observ.). However, the study by Stabenau *et al.* (1991) did not address the physiological effects of multiple submergence of sea turtles. More recently, Stabenau and Vietti (1998) investigated the

effects of three successive submergence episodes of loggerhead sea turtles with an interval of 10, 42 or 180 min between each 7.5 min submergence period. The data revealed that the initial submergence produced a significant and severe acid-base disturbance. Successive submergence episodes caused decreases in blood pH, and increases in blood Pco_2 and lactate that were less in magnitude than that measured following the initial submergence. In addition, more than three hours was required to completely recover from the metabolic derangement. Overall, the data suggested that while repeated submergence induces significant blood acid-base disturbances, repetitive submergence should not limit sea turtle survival potential given adequate recovery time at the surface. One problem with the experimental design of the laboratory multiple submergence experiment was that it was conducted under controlled conditions, whereby the turtles were placed into a canvass bag to minimize struggling during submergence. This methodology minimized potential sea turtle mortality, but may have led to an underestimate of the acid-base imbalance caused by the multiple submergence. Therefore, the purpose of the present study was to examine the physiological effects of multiple forced submergence of loggerhead sea turtles during exposure in TED-equipped commercial fishing nets. These data may offer greater insight into potential sea turtle mortality caused by multiple capture in commercial shrimping nets carrying legal TEDs.

Materials and Methods

Thirty-six 2-year old loggerhead sea turtles from the NMFS Galveston Laboratory were used in this study. The turtles were transported from Galveston, TX to Panama City, FL where they were placed into pens in St. Andrews Bay. Each turtle was randomly placed into the experimental (submerged) or control (non-submerged) treatment. Table 1 lists the straight standard carapace length and weight of the 24 experimental turtles used in the submergence study, while Table 2 lists the morphometric data for the 12 control turtles. All turtles were of comparable size and weight, and therefore, any alterations in blood parameters between experimental and control turtles represented treatment effects rather than size effects. The submergence study was initiated after approximately 21 days of natural conditioning in the in-water pens.

The study was initiated by collecting pre-submergence blood samples from the experimental turtles immediately prior to their individual confinement in a weighted mesh bag. Each turtle was then submerged using the standard protocol for TED certification tests. Briefly, the mesh bag containing a turtle was placed onto a line connecting the trawl vessel to the headrope on the shrimp net. Divers then released the turtle (without handling the animal) into the mouth of the trawl. Although the shrimp net was equipped with a TED, divers held the escape door closed for 5 min. The turtle was then permitted to leave the trawl and surface. Thus, the total submergence time was approximately 7.5 min, including the time for the weighted mesh bag containing the turtle to reach the headrope for release into the trawl, the 5 min within the trawl, and the time for the turtle to surface. Turtles were immediately captured at the surface and returned to the trawl vessel for post-

submergence blood sampling. Typically, post-submergence blood samples were collected within 1 min of the turtle surfacing. Following a rest interval of 10 (treatment 1), 42 (treatment 2) or 180 (treatment 3) min in water-filled containers on the trawl vessel, a pre-submergence blood sample was collected and the turtle was submerged a second time. A post-submergence blood sample was then collected immediately upon surfacing. The turtle was then submerged a third time, following the same rest interval between the first and second submergence episodes, and pre- and post-submergence blood samples were collected as described above. A final blood sample was collected 180 min after the final submergence in all turtles. Blood samples were also collected from non-submerged control turtles over the same time intervals to ensure that repetitive handling and blood sampling did not alter blood homeostasis. All blood samples were collected into heparinized vacutainers from the dorsal cervical sinus as described by Owens and Ruiz (1980). No more than 4-6% of blood volume was collected during the serial sampling to minimize potential physiological affects associated with blood volume depletion.

Blood gases (Po_2 and Pco_2) and pH were analyzed immediately following collection using a blood gas analyzer thermostatted to turtle body temperature (27-28.5 °C). Packed red cell volume (hematocrit) was determined following centrifugation of heparinized micro-capillary tubes. Two hundred microliters of whole blood were then added to 10% trichloroacetic acid for lactate analysis. The deproteinized samples were centrifuged, and the supernatant removed and stored at -70°C. Lactate was determined spectrophotometrically using standard enzymatic techniques (Sigma, kit 826-B). The remaining whole blood was then centrifuged, the plasma removed and stored at -70°C.

Plasma Na^+ and K^+ were measured with flame photometry (Jenway, Model PFP7), while plasma Cl^- was determined with electrometric titration (Haake-Bucher, Model 4425000). Plasma osmolality was determined with a vapor pressure osmometer (Wescor, Model 5500). Plasma catecholamine (norepinephrine and epinephrine) samples have been processed and extracted. The samples will be analyzed with high-pressure liquid chromatography (BAS, Model 480). Catecholamine data will be provided to the NMFS Galveston Laboratory as soon as the analyses are completed. However, the preliminary data suggest that catecholamine concentrations recover more rapidly from acid-base imbalances than respiratory and metabolic components.

All data are expressed as means ± SE. Where appropriate, the data was analyzed with one-way ANOVA. Post-hoc comparisons between means were analyzed with Tukey's multiple comparison test. A fiduciary level of $P \leq 0.05$ was regarded as significant.

Results

Blood acid-base status. The initial submergence of loggerhead sea turtles produced a dramatic and severe acidosis in all experimental turtles with blood pH falling an average of 0.63 ± 0.06 (range 0.53 to 0.73 pH units) from pre-submergence values (Figure 1). The blood acidosis was derived from respiratory and metabolic components as evident from a positive proton-lactate deficit (Buffer capacity • ΔpH - Δ[lactate]), and from significant increases in blood P_{CO_2} (average increase 45 ± 3 mm Hg, Figure 2) and blood lactate (average increase 10.13 ± 0.6 mM, Figure 3) in all experimental turtles. Significant decreases in blood P_{O_2} also occurred following the initial submergence (Figure 4). In contrast, no significant changes in blood pH, P_{CO_2} and P_{O_2}, and lactate were measured in non-submerged control turtles (Tables 3-5) following collection of the first two samples.

Recovery of the respiratory and metabolic imbalance in submerged turtles was dependent on the interval between successive submergences. A 10 min interval between the first and second submergence (treatment 1 turtles; blood collection 3 on the figures) permitted partial recovery of blood pH (Figure 1A), although these values remained significantly lower than the pre-submergence values. The blood P_{CO_2} and P_{O_2} were comparable to the initial pre-submergence values (Figures 2A and 4A). However, additional increases in the blood lactate concentration were measured during the first recovery interval in these turtles (Figure 3A). Turtles with a 42 min interval (treatment 2 turtles) between the first and second submergence had complete recovery of blood pH (Figure 1B), P_{CO_2} (Figure 2B), P_{O_2} (Figure 4B), and slight recovery of the blood lactate concentration (Figure 3B). The [lactate] in the third blood sample from treatment 2

turtles remained significantly elevated from the pre-submergence values. Turtles with a 180 min interval (treatment 3 turtles) between the first and second submergence had complete recovery of the blood pH, Pco_2, Po_2 and lactate (Figures 1C-4C). Non-submerged control turtles exhibited no significant changes in blood pH, Pco_2, and Po_2, whether the interval between collection of the second and third serial blood sample was 10, 42 or 180 min (Tables 3-5). The lactate concentration in control turtles was not affected by repetitive handling (Tables 3-5), although the [lactate] decreased significantly from the initial blood sample lactate values in one control group (Table 5).

The second 7.5 min submergence produced a significant decrement in blood pH and increment in Pco_2 (Figure 1-2) in all experimental treatments. However, the severity of the acid-base imbalance was not as drastic as the acidosis measured following the first submergence. The mean pH difference (Δ pH) between the second pre- and post-submergence blood samples ranged from 0.16 in treatment 1 turtles to 0.66 in treatment 3 turtles. The substantial drop in blood pH in treatment 3 turtles resulted from greater pre- to post-submergence increases in blood Pco_2 and lactate (Figures 2-3) than measured in treatment 1 and 2 turtles. Treatment 3 turtles had a comparable acid-base response to that measured following the initial submergence, whereas treatment 1 and 2 turtles had a reduced acid-base deficit as a result of retention of blood lactate during the recovery period. Collection of the fourth sample from non-submerged control turtles revealed no significant changes in the blood pH, Pco_2, Po_2 or lactate concentration (Tables 3-5).

The fifth serial blood sample collected from treatment 1 and 2 turtles revealed that the blood pH remained significantly lower than the initial pre-submergence value (Figure 1A), reflecting the brief 10 min or 42 min post-submergence recovery interval, respectively, following the second submergence. The acidosis in these animals was due to the continued presence of blood lactate during the post-submergence recovery period (Figures 3A and 3B). In contrast, the blood pH, P_{CO_2}, P_{O_2} and [lactate] were completely recovered in treatment 3 turtles as a result of the 3 h post-submergence recovery interval (Figures 1C-4C). The acid-base status of non-submerged control turtles was unaffected by collection of the fifth serial blood sample (Tables 3-5).

The third and final submergence produced comparable acid-base changes in treatment 1-3 turtles to that measured following the second submergence. The Δ pH ranged from 0.11 in treatment 1 turtles to 0.65 in treatment 3 turtles (Figure 1). Blood P_{CO_2} increased in all experimental turtles following the third submergence, with increments ranging from 9.3 to 27.5 mm Hg in treatment 1 and 3 turtles, respectively (Figure 2). Lactate also increased in all experimental turtles following the third submergence, with the magnitude of the increases ranging from 0.9 mM in treatment 1 turtles to 9.3 mM in treatment 3 turtles (Figure 3). In all experimental turtles, the third submergence produced significant decreases in the blood P_{O_2} (Figure 4). No blood acid-base changes were measured in non-submerged control turtles following collection of the sixth serial sample (Tables 3-5).

Blood homeostasis was achieved in all experimental turtles 3 h after the final forced submergence (Figures 1-3). No significant changes in the blood pH, Pco_2, Po_2 and lactate concentration were detected when compared to the initial pre-submergence values in treatment 1-3 turtles. Similarly, few significant changes in the blood acid-base status were measured in non-submerged control turtles following collection of the final serial sample (Tables 3-5).

Plasma ion concentration and osmotic pressure. Brief forced submergence of loggerhead turtles had a profound effect on the plasma ionic status. Plasma $[K^+]$ increased significantly immediately following submergence in all experimental turtles. Significant increases were also observed in the plasma $[Na^+]$ and osmotic pressure, although these changes did not occur in turtles from all of the experimental treatments (Tables 6-8). Turtles recovered from the ionic imbalances in turtles with a 42 min or 3 h post-submergence interval between collection of the second and third blood sample. However, the plasma K^+ remained significantly higher in turtles with a 10 min post-submergence recovery interval. The second submergence also caused significant increases in plasma K^+ in all experimental turtles (Tables 6-8), whereas the plasma $[Cl^-]$ and $[Na^+]$ were unaffected. As before, the ionic disturbances were resolved during the post-submergence recovery interval, with the exception of the significantly elevated plasma K^+ in turtles with a 10 min post-submergence recovery interval (Tables 6-8). The plasma ionic concentrations and osmotic pressure were not significantly different in treatment 1 and 3 turtles following the third submergence (Tables 6 and 8). However, significant increases in the plasma Na^+ and K^- concentrations were measured in treatment 2 turtles following

the final submergence (Table 7). The ionic concentrations in all experimental turtles 3 h following the final submergence were comparable to the initial pre-submergence values (Tables 6-8). Finally, it should be noted that the plasma ion concentrations and osmotic pressure in non-submerged control turtles were unaffected by serial blood sampling (Tables 9-11). These data suggest that ionic changes in experimental turtles were due to the forced submergence and was not an artifact of handling and repetitive blood sampling.

Discussion

Acid-Base Status. Brief submergence (7.5 min) of 2-yr old loggerhead sea turtles in commercial fishing nets produced a severe and significant acid-base imbalance, whereby pH dropped an average of 0.63 ± 0.06 U after the initial forced submergence. For comparison, a pH drop ranging from 0.37 to 0.50 has been measured in Kemp's ridley (*Lepidochelys kempi*) and loggerhead sea turtles following trawling (Stabenau *et al.*, 1991; Stabenau and Vietti, 1999). Turtles in the latter studies were permitted to escape the TED, and thus, the total submergence duration averaged < 3 to 4 min. To our knowledge, Stabenau and Vietti (1998) have conducted the only study in the literature that offers a comparable experimental protocol to that reported herein. Those authors reported a pH decrease of 0.54 ± 0.03 U following 7.5 min of confined forced submergence of 2-yr old loggerhead turtles under laboratory conditions. The greater acidosis measured in trawled turtles in the study herein resulted from increased swimming activity during the forced submergence. This is confirmed by a post-submergence increase in blood lactate of 10.1 mM under trawling conditions versus 8.8 mM following laboratory submergence.

Recovery of blood homeostasis was dependent on the length of the interval between submersion episodes. Turtles with a 10 min interval following submersion had a lower pH, higher P_{CO_2} and increased lactate than turtles with a 42 or 180 min post-submergence recovery interval. These results were comparable to those reported for forcibly submerged loggerhead turtles by Stabenau and Vietti (1998). Turtles forcibly

submerged under lab or field conditions hyperventilate upon surfacing. Stabenau *et* al. (1991) reported a 9- to 10-fold increase in the breathing frequency of trawled Kemp's ridley turtles. Comparable breathing rates were observed in the present study (data not shown) after submersion. Thus, turtles with a brief period between the submergence episodes would have a limited ability to release the CO_2 retained during submersion or to breakdown lactic acid produced during the course of the forced dive. In fact, blood [lactate] continued to increase in treatment 1 turtles during collection of the first six serial samples. Substantial retention of CO_2 and lactate during the 10 min post-submergence recovery interval reduced blood pH when compared to the other two treatments. In contrast, turtles with a 42 or 180 min interval between the submersion episodes would have had more time to eliminate CO_2 and lactate during the recovery intervals. Treatment 2 turtles exhibited a 6% drop in the blood [lactate] during the first 42 min post-submergence recovery interval and a 17.5% decrease in the blood [lactate] during the second recovery interval. Thus, a 42 min post-submersion recovery interval permitted recovery of blood gases, but was inadequate to clear blood lactate. Lactate declined 80.4% and 83.8%, respectively, during the first two 180 min post-submergence recovery intervals in treatment 3 turtles. Therefore, the longer surface interval resulted in an increased ability to recover from the submersion episodes. In fact, lactate declined 82.7%, 82.8% and 87.9%, respectively, in treatment 1, 2 and 3 turtles 180 min after the final submersion episode.

Lutz and Dunbar-Cooper (1987) reported that loggerhead turtles captured during trawling at Cape Canaveral, Florida exhibited a 16.8% decline in lactate 3 hr following

submergence. Those authors proposed that the rate of lactate decline was dependent on the magnitude of the lactate concentration, so that 10 mM of lactate would decline at a rate of 1.25 mM lactate hr^{-1}. However, in the current study the rate of lactate decline was considerably higher than suggested by Lutz and Dunbar-Cooper (1987). The overall rate of lactate decline in the present study was 3.6 ± 0.2 mM hr^{-1}. For comparison, Stabenau and Vietti (1998) reported an overall rate of lactate decline of 2.6 ± 0.2 mM hr^{-1} in loggerheads forcibly submerged 7.5 min under laboratory conditions.

Ions, Osmolality and Hematocrit. There are three primary mechanisms for recovery of blood pH following an acid-base disturbance: cellular buffering, and respiratory and renal compensation. Cellular responses occur immediately following the disturbance, whereas respiratory and renal adjustments occur within minutes to hours, respectively. Previously, Stabenau *et al.* (1991) and Stabenau and Vietti (1998) have reported that Kemp's ridley and loggerhead sea turtles exhibited a significant increase in plasma $[K^+]$ following submergence. In the present study, a cellular response to the severe acid-base disturbance caused by multiple forced submergence was suggested by alterations of the plasma ion concentrations and osmolality during and after trawling. Correlation analyses confirmed that decreases in blood pH were associated with increases in the plasma ion concentrations and osmolality (data not shown). Stabenau and Vietti (1998) suggested that turtles might possess stress-mediated red blood cell ion transporters that are activated to restore cell volume and/or cellular pH. The presence of ion transporters would explain the changes to plasma ion concentrations and osmolality during acidosis and warrants further investigation.

Effects of Handling. The blood samples from control turtles did not exhibit significant changes in the blood pH, Pco_2, Po_2, [lactate], or ion concentrations. These data suggest that repetitive serial blood sampling did not cause alterations to acid-base and ionic status in loggerhead sea turtles. Stabenau and Vietti (1998) reached a comparable conclusion following serial sampling of control loggerhead turtles. Thus, the changes in the blood parameters measured in the experimental turtles in the present study were the result of the forced submergence and not an artifact of handling.

Conclusions. From the current study, the data suggest that forced submergence of 2-yr old loggerhead sea turtles produces a significant blood metabolic and respiratory acidosis. Repetitive submergence did not augment the acidosis rather subsequent submergence resulted in less severe acid-base disturbances. Under trawl conditions, the turtle must recover from any physiological acid-base disturbance when it is freed from a TED-equipped net. This is accomplished, in part, by the turtle immediately surfacing and hyperventilating (Jackson, 1985, Stabenau *et al.*, 1991). This behavior was observed during the current study following each submergence episode. Turtles would resume normal voluntary diving behavior, presumably after partial to complete recovery from the acid-base disturbance. These data suggest that repetitive submergence of sea turtles in TEDs would not significantly affect their survival potential, provided that the turtles have an adequate recovery surface interval between successive submergences. However, it should be noted that the latter statement is based on turtles that may be involuntarily submerged in shrimp nets equipped with legally certified and installed turtle excluder devices. Poor installation or lack of use of legal TEDs would result in augmenting the

acid-base imbalance in the turtles. Increasing the magnitude of the blood acid-base and ionic disturbance during each submersion would increase the length of time necessary to achieve partial or complete recovery.

Acknowledgments

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Table 1. Straight standard carapace length, weight, and the interval between successive submergence episodes for experimental turtles.

Tag #	Submergence Interval	Length (cm)	Weight (kg)
sss-404	10 min	36.3	7.19
sss-408	10 min	36.3	6.69
sss-441	10 min	36.2	6.73
sss-456	10 min	34.5	6.19
sss-495	10 min	36.6	7.20
sss-503	10 min	35.0	6.30
sss-559	10 min	35.9	6.85
sss-564	10 min	35.0	6.01
	mean	35.7	6.64
	SE	0.3	0.16
sss-448	42 min	34.7	5.92
sss-479	42 min	35.4	5.99
sss-490	42 min	37.1	7.15
sss-505	42 min	37.0	7.53
sss-509	42 min	37.10	7.10
sss-553	42 min	35.0	6.42
sss-562	42 min	36.4	6.69
sss-588	42 min	36.0	6.82
	mean	36.1	6.70
	SE	0.3	0.20
sss-445	180 min	34.6	6.25
sss-453	180 min	36.5	7.19
sss-466	180 min	33.9	7.15
sss-523	180 min	34.4	6.82
sss-534	180 min	36.4	7.07
sss-541	180 min	36.6	6.89
sss-567	180 min	36.3	7.06
sss-576	180 min	37.4	7.36
	mean	35.8	6.97
	SE	0.4	0.12

Table 2. Straight standard carapace length and weight of individual control turtles.

Tag #	Length (cm)	Weight (kg)
sss-491	35.5	6.18
sss-528	34.4	5.99
sss-565	34.5	6.53
sss-574	35.2	6.11
sss-407	36.0	6.65
sss-422	34.5	6.11
sss-513	36.5	7.00
sss-514	37.0	7.15
sss-485	36.7	6.72
sss-536	36.5	6.90
sss-593	34.7	6.07
sss-598	33.9	6.11
Mean	35.4	6.46
SE	0.3	0.12

Table 3. Blood pH, Pco_2, Po_2, and lactate from control non-submerged turtles to examine the effects of repetitive sampling. Serial blood samples were collected with a 7.5 min interval between samples 1-2, 3-4, and 5-6. A 10 min interval separated collection of samples 2-3, and 4-5. Sample 7 was collected 3 h after sample 6. A significant difference between samples 1-2, 3-4, and 5-6 are indicated by an asterisk (*), whereas significant differences of samples from the initial pre-submergence sample (serial sample 1) are denoted by a pound sign (#).

Serial Sample	pH	Pco_2 (mm Hg)	Po_2 (mm Hg)	Lactate (mM)
1	7.54 ± 0.02	30.2 ± 1.5	58.9 ± 5.3	0.5 ± 0.1
2	7.55 ± 0.02	28.2 ± 2.3	73.2 ± 3.0	0.6 ± 0.0
3	7.56 ± 0.03	30.4 ± 2.4	73.3 ± 3.7	1.0 ± 0.2
4	7.58 ± 0.02	31.2 ± 1.9	70.6 ± 5.4	1.1 ± 0.1
5	7.55 ± 0.02	30.9 ± 2.4	61.8 ± 6.8	1.1 ± 0.2
6	7.53 ± 0.02	31.2 ± 2.0	68.2 ± 6.2	1.3 ± 0.2#
7	7.53 ± 0.03	33.8 ± 1.9	58.4 ± 1.6	0.7 ± 0.1

Table 4. Blood pH, Pco_2, Pco_2, and lactate from control non-submerged turtles to examine the effects of repetitive sampling. The rest of the legend is as in Table 3, with the exception that the interval between samples 2-3, and 4-5 was 42 min.

Serial Sample	pH	Pco_2 (mm Hg)	Po_2 (mm Hg)	Lactate (mM)
1	7.50 ± 0.01	37.2 ± 1.5	67.6 ± 6.5	0.9 ± 0.1
2	7.52 ± 0.02	34.9 ± 1.8	68.2 ± 5.3	1.1 ± 0.1
3	7.58 ± 0.02	32.8 ± 1.5	64.0 ± 3.5	0.8 ± 0.1
4	7.55 ± 0.03	36.5 ± 3.2	64.2 ± 3.7	1.6 ± 0.5
5	7.60 ± 0.01#	33.1 ± 0.7	61.4 ± 4.1	0.9 ± 0.2
6	7.60 ± 0.02#	32.9 ± 0.6	65.9 ± 1.6	1.1 ± 0.2
7	7.54 ± 0.02	30.7 ± 0.8	59.9 ± 3.0	0.7 ± 0.0

Table 5. Blood pH, Pco_2, Po_2, and lactate from control non-submerged turtles to examine the effects of repetitive sampling. The rest of the legend is as in Table 3, with the exception that the interval between samples 2-3, and 4-5 was 180 min.

Serial Sample	pH	Pco_2 (mm Hg)	Po_2 (mm Hg)	Lactate (mM)
1	7.47 ± 0.03	28.6 ± 0.8	68.9 ± 1.9	1.5 ± 0.2
2	7.47 ± 0.02	31.0 ± 1.7	68.5 ± 3.9	1.1 ± 0.2
3	7.52 ± 0.01	29.2 ± 1.6	60.1 ± 3.5	0.6 ± 0.1#
4	7.54 ± 0.02	29.2 ± 0.4	63.3 ± 4.4	0.9 ± 0.1
5	7.56 ± 0.01#	28.0 ± 1.8	64.9 ± 3.2	0.6 ± 0.2#
6	7.54 ± 0.03	32.6 ± 1.4	73.3 ± 2.6	0.9 ± 0.2
7	7.52 ± 0.01	34.4 ± 0.8	61.4 ± 2.1	0.7 ± 0.1#

Table 6. Mean (± SE) plasma Na^+, K^+, Cl^- concentration, hematocrit and plasma osmotic pressure prior to and following multiple forced submergence in loggerhead sea turtles. The interval between each submergence episode was 10 min. The rest of the legend as in Table 3.

Serial Sample	Na^+ (mM)	K^+ (mM)	Cl^- (mM)	Hematocrit (%)	Osmotic pressure (mosmoles•kg^{-1})
1	153 ± 2	3.3 ± 0.3	112 ± 5	31.9 ± 1.2	318 ± 4
2	171 ± 8	5.5 ± 0.3*#	120 ± 3	32.6 ± 0.7	345 ± 4*#
3	156 ± 6	4.3 ± 0.0#	113 ± 6	32.4 ± 0.6	332 ± 4
4	171 ± 8	5.3 ± 0.1*#	123 ± 3	32.9 ± 0.7	349 ± 1#
5	166 ± 4	4.3 ± 0.1#	114 ± 3	32.6 ± 0.9	334 ± 2
6	166 ± 12	5.1 ± 0.1#	116 ± 5	31.9 ± 1.1	335 ±11
7	157 ± 4	3.7 ± 0.1	111 ± 3	29.5 ± 1.1	325 ± 2

Table 7. Mean (± SE) plasma Na^+, K^+, Cl^- concentration, hematocrit, and plasma osmotic pressure prior to and following multiple forced submergence in loggerhead sea turtles. The interval between each submergence episode was 42 min. The rest of the caption is as described in Table 3.

Serial Sample	Na^+ (mM)	K^+ (mM)	Cl^- (mM)	Hematocrit (%)	Osmotic pressure (mosmoles•kg^{-1})
1	160 ± 4	3.1 ± 0.2	112 ± 3	29.6 ± 1.1	331 ± 12
2	186 ± 8*#	5.0 ± 0.4*#	120 ± 2	31.4 ± 1.1	368 ± 10
3	163 ± 3	2.8 ± 0.1	112 ± 4	29.6 ± 1.3	338 ± 11
4	181 ± 3	4.9 ± 0.3*#	120 ± 4	29.1 ± 1.1	361 ±13
5	160 ± 8	2.9 ± 0.2	115 ± 6	28.7 ± 1.0	332 ± 9
6	185 ± 4*#	4.5 ± 0.4*#	114 ± 1	28.7 ± 1.0	343 ±14
7	161 ± 6	2.6 ± 0.2	108 ± 3	29.5 ± 1.1	326 ± 9

Table 8. Mean (± SE) plasma Na^+, K^+, Cl^- concentration, hematocrit, and plasma osmotic pressure prior to and following multiple forced submergence in loggerhead sea turtles. The interval between each submergence episode was 180 min. The rest of the caption is as described in Table 3.

Serial Sample	Na^+ (mM)	K^+ (mM)	Cl^- (mM)	Hematocrit (%)	Osmotic pressure (mosmoles•kg^{-1})
1	164 ± 2	4.5 ± 0.7	114 ± 3	31.4 ± 1.9	325 ± 9
2	188 ± 4	7.0 ± 0.6*#	125 ± 5	31.2 ± 1.0	355 ± 3*#
3	163 ± 10	3.6 ± 0.3	116 ± 4	29.7 ± 0.4	314 ± 3
4	176 ± 10	6.2 ± 0.3*#	116 ± 7	29.0 ± 1.1	352 ± 9*
5	173 ± 10	4.0 ± 0.2	117 ± 5	27.2 ± 0.3	323 ± 3
6	175 ± 18	5.3 ± 0.0	112 ± 5	28.9 ± 0.6	333 ± 11
7	159 ± 11	3.6 ± 0.6	116 ± 7	25.3 ± 0.5	320 ± 4

Table 9. Mean (± SE) plasma Na^+, K^+, and Cl^- concentration, and plasma osmotic pressure in control non-submerged turtles. Samples were taken following the 10 min blood collection protocol. None of the pre- to post-submergence samples was significantly different.

Serial Sample	Na^+ (mM)	K^+ (mM)	Cl^- (mM)	Osmotic pressure (mosmoles•kg^{-1})
1	150 ± 3	3.0 ± 0.2	116 ± 2	313 ± 8
2	144 ± 6	3.2 ± 0.1	110 ± 2	309 ± 6
3	146 ± 2	3.1 ± 0.1	112 ± 2	314 ± 7
4	153 ± 1	3.2 ± 0.1	112 ± 3	314 ± 4
5	150 ± 3	3.2 ± 0.2	111 ± 4	324 ± 4
6	143 ± 4	3.2 ± 0.1	114 ± 5	313 ± 4
7	142 ± 4	3.1 ± 0.2	109 ± 2	315 ± 7

Table 10. Mean (± SE) plasma Na^+, K^+, and Cl^- concentration, and plasma osmotic pressure in control non-submerged turtles. Samples were taken following the 42 min blood collection protocol. None of the pre- to post-submergence samples was significantly different.

Serial Sampling	Na^+ (mM)	K^+ (mM)	Cl^- (mM)	Osmotic pressure (mosmoles•kg^{-1})
1	139 ± 6	3.4 ± 0.2	115 ± 2	321 ± 7
2	146 ± 5	3.2 ± 0.1	116 ± 2	314 ± 7
3	146 ± 4	3.4 ± 0.2	116 ± 2	316 ± 6
4	144 ± 4	3.4 ± 0.2	110 ± 2	319 ± 5
5	145 ± 3	3.3 ± 0.2	114 ± 3	315 ± 4
6	146 ± 6	3.3 ± 0.3	114 ± 3	317 ± 5
7	148 ± 3	3.2 ± 0.2	114 ± 2	319 ± 6

Table 11. Mean (± SE) plasma Na^+, K^+, and Cl^- concentration, and plasma osmotic pressure in control non-submerged turtles. Samples were taken following the 180 min blood collection protocol. None of the pre- to post-submergence samples was significantly different.

Serial Sample	Na^+ (mM)	K^+ (mM)	Cl^- (mM)	Osmotic pressure (mosmoles•kg^{-1})
1	151 ± 1	3.1 ± 0.1	113 ± 2	310 ± 5
2	152 ± 5	3.1 ± 0.1	115 ± 1	311 ± 6
3	156 ± 2	3.0 ± 0.1	117 ± 4	310 ± 6
4	148 ± 3	3.0 ± 0.1	116 ± 1	311 ± 5
5	151 ± 4	2.9 ± 0.1	117 ± 1	307 ± 5
6	146 ± 2	2.9 ± 0.1	118 ± 2	308 ± 5
7	154 ± 5	2.9 ± 0.2	119 ± 3	307 ± 8

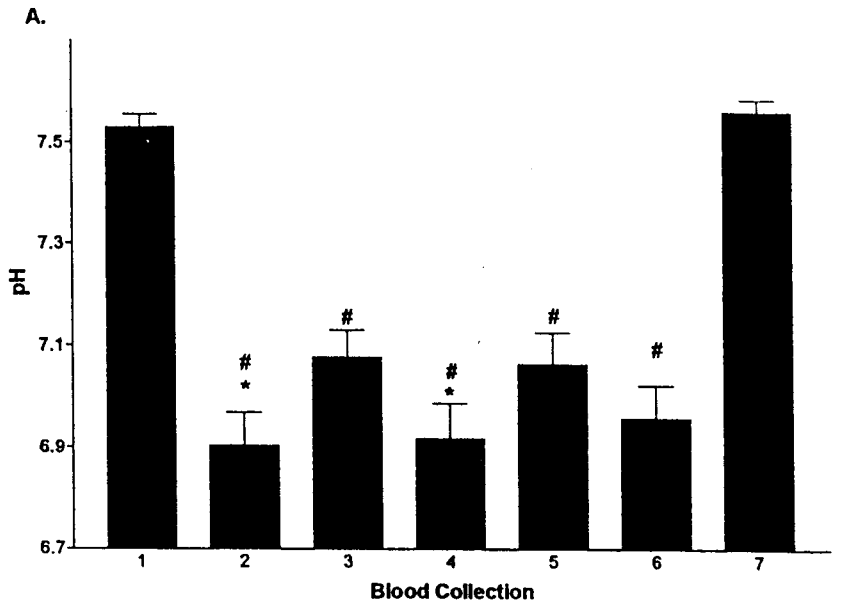
Figure Legends

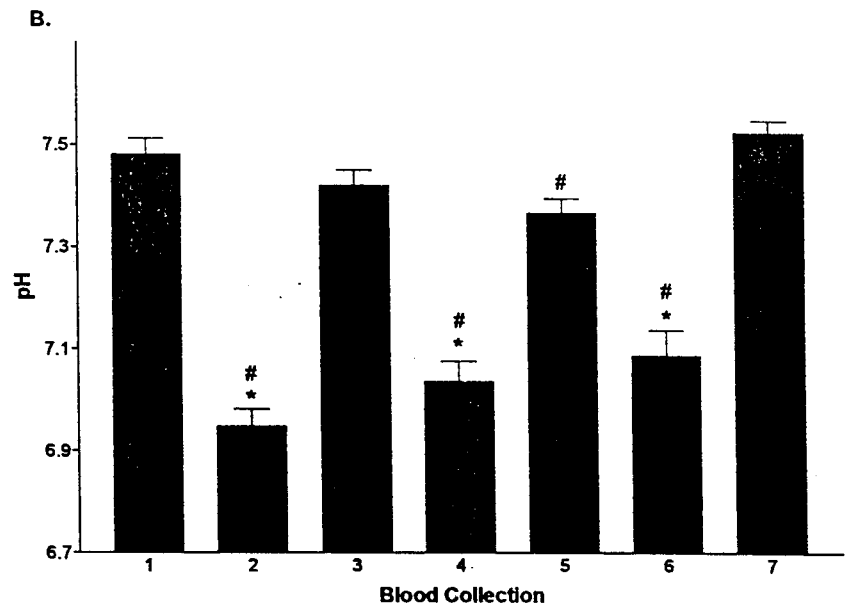
1. Blood pH measured prior to and after three successive forced submergence episodes in loggerhead sea turtles. Samples 1, 3 and 5 are pre-submergence, whereas samples 2, 4, and 6 are post-submergence. Sample 7 was collected three hours after the final submergence. The interval between the submergences was 10 min (A), 42 min (B) or 180 min (C).

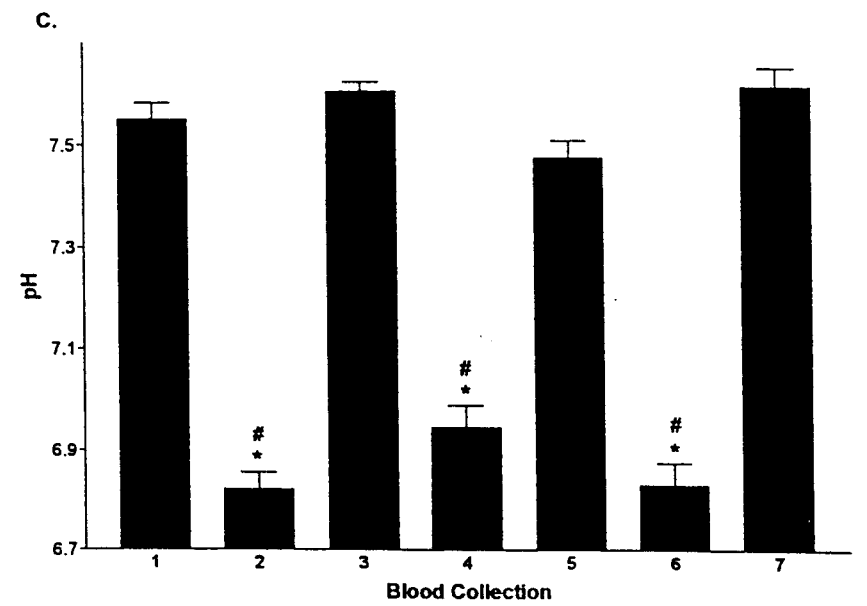
2. Blood Pco_2 measured prior to and after three successive forced submergence episodes in loggerhead sea turtles. The interval between the submergences was 10 min (A), 42 min (B) or 180 min (C). Rest of the legend as in Figure 1.

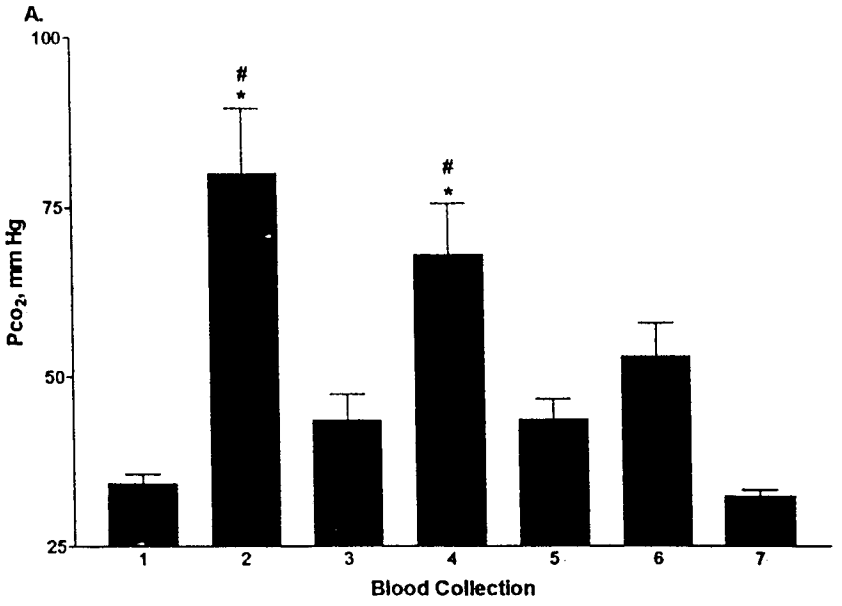
3. Blood lactate concentration measured prior to and after three successive forced submergence episodes in loggerhead sea turtles. The interval between the submergences was 10 min (A), 42 min (B) or 180 min (C). Rest of the legend as in Figure 1.

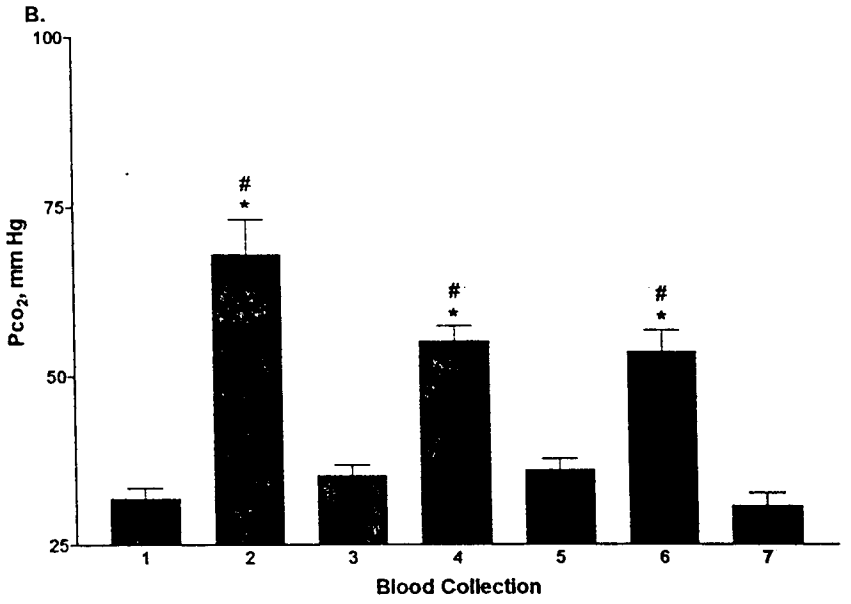
4. Blood Po_2 measured prior to and after three successive forced submergence episodes in loggerhead sea turtles. The interval between the submergences was 10 min (A), 42 min (B) or 180 min (C). Rest of the legend as in Figure 1.

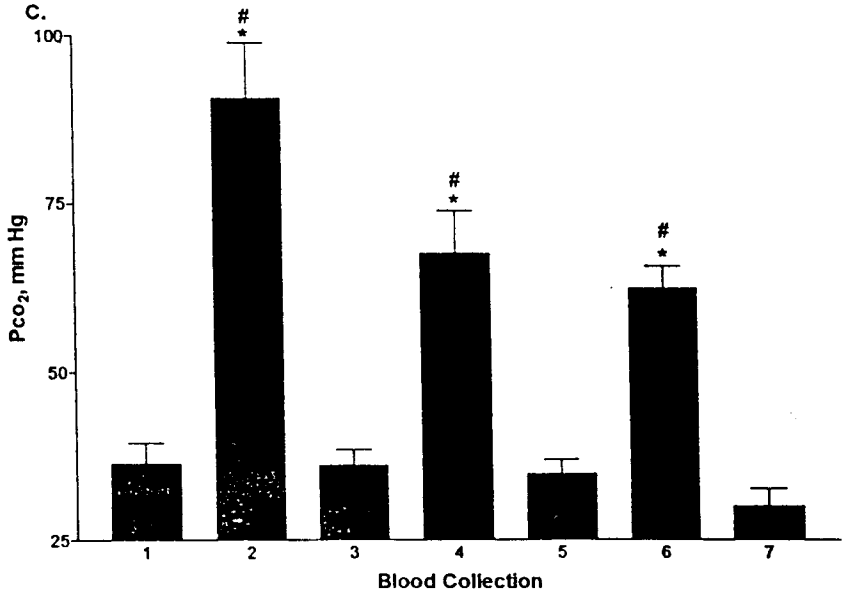

A.
pH
7.5
7.3
7.1
6.9
6.7
1
2
3
4
5
6
7
Blood Collection

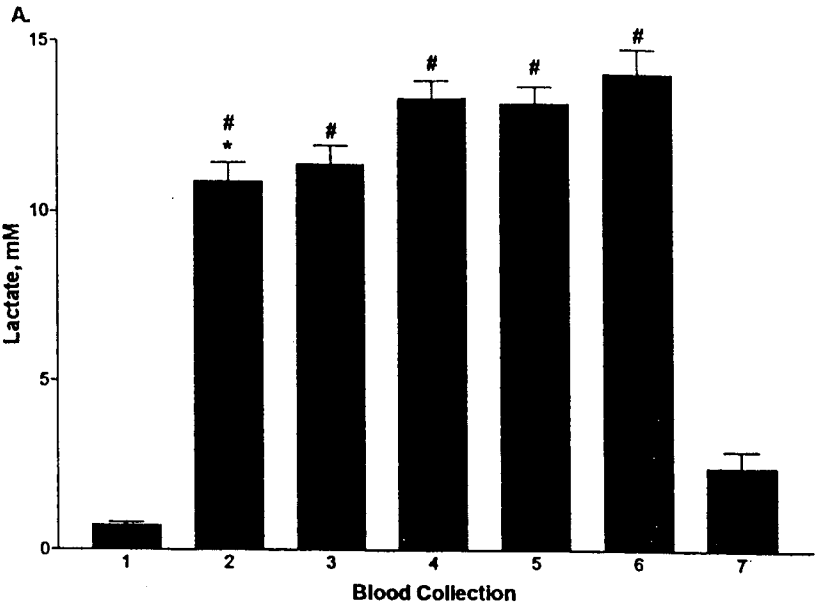

B.
pH
7.5
7.3
7.1
6.9
6.7
1
2
3
4
5
6
7
Blood Collection

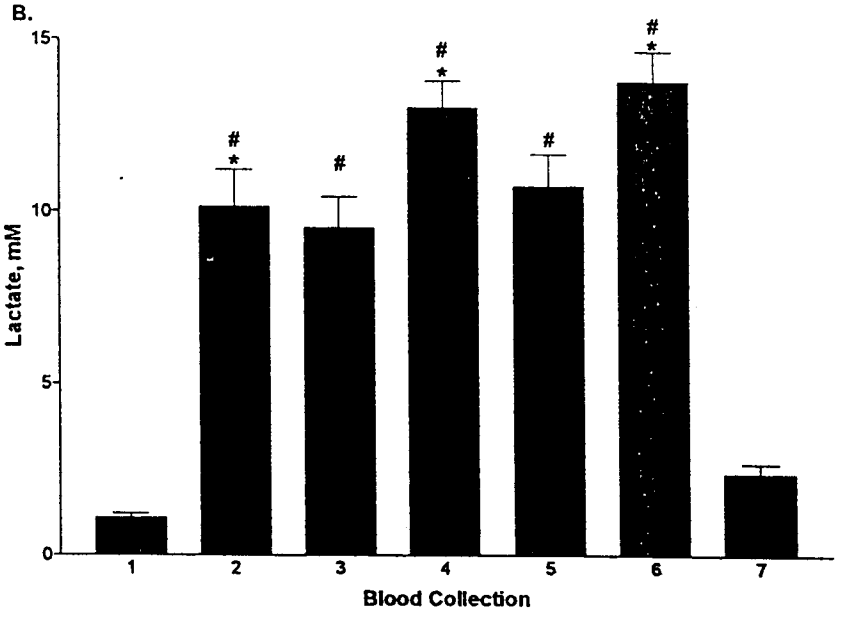

C.
pH
7.5
7.3
7.1
6.9
6.7
1
2
3
4
5
6
7
Blood Collection

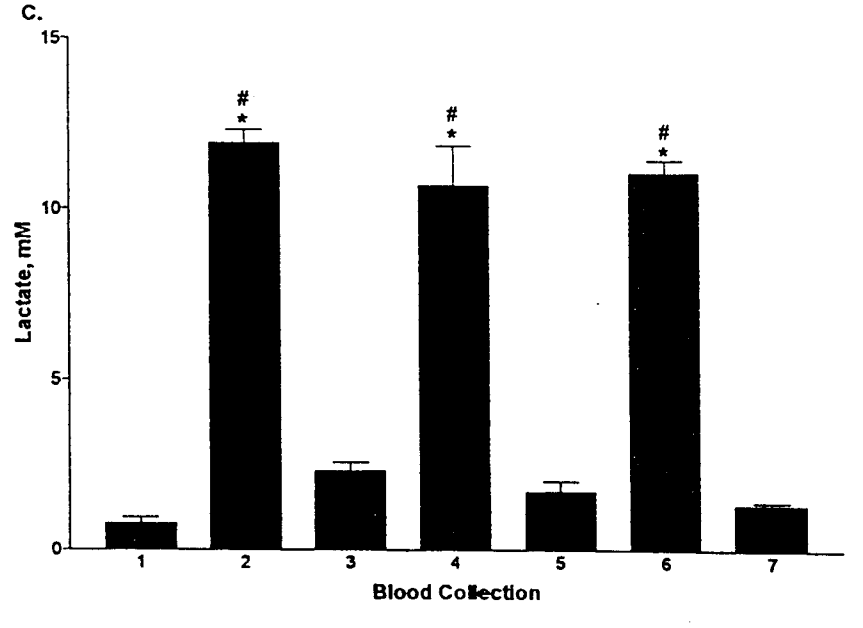

A.
100
75
50
25
Pco_2, mm Hg
#
*
1
2
3
4
5
6
7
Blood Collection

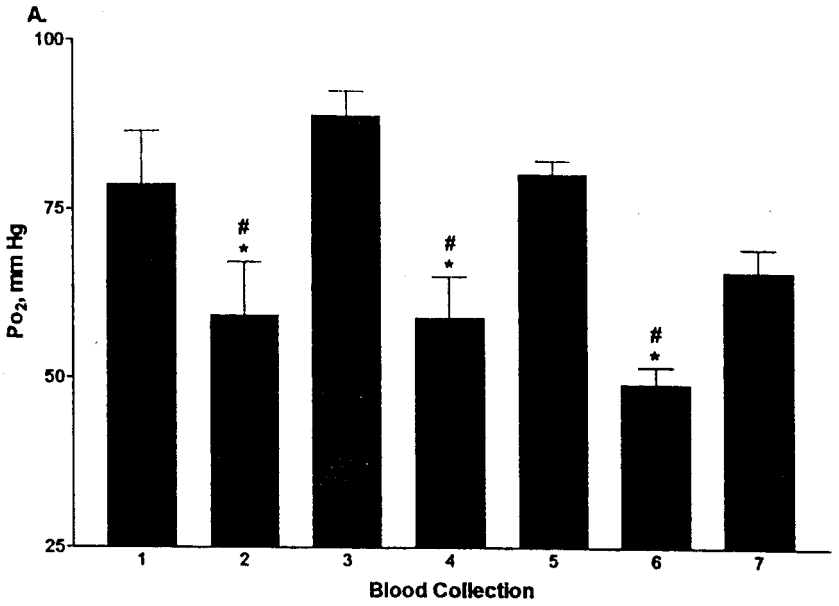

B.
100
75
50
25
Pco_2, mm Hg
#
*
1
2
3
4
5
6
7
Blood Collection

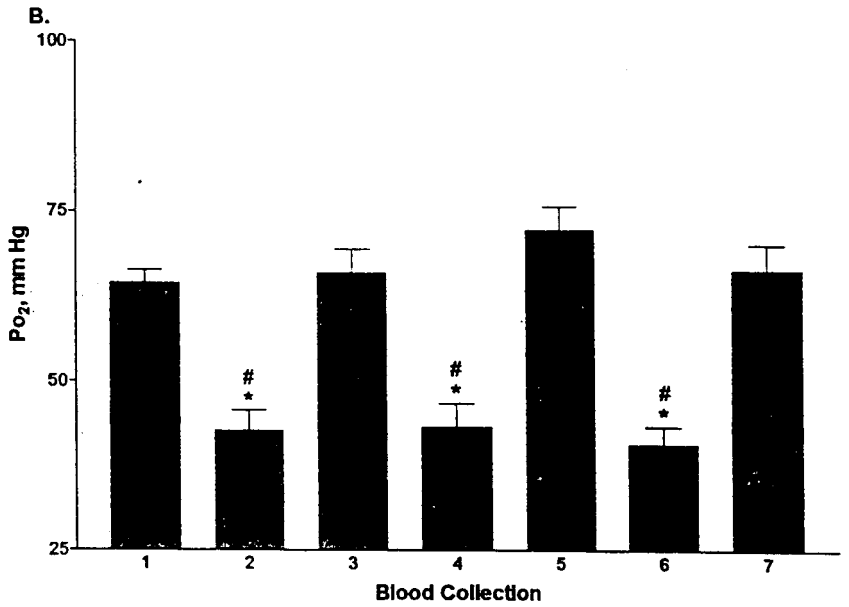

C.
100
75
50
25
Pco_2, mm Hg
#
*
1
2
3
4
5
6
7
Blood Collection

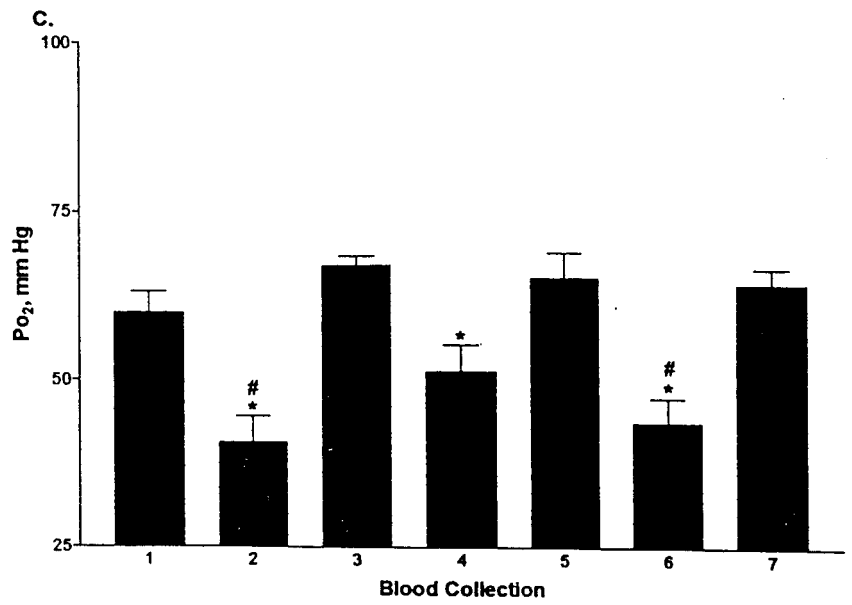

A.
Lactate, mM
15
10
5
0
1
2
3
4
5
6
7
Blood Collection


B.
Lactate, mM
15
10
5
0
1
2
3
4
5
6
7
Blood Collection


C.
Lactate, mM
15
10
5
0
1
2
3
4
5
6
7
Blood Collection


A.
100
75
50
25
Po_2, mm Hg
#
*
1
2
3
4
5
6
7
Blood Collection


B.
100
75
50
25
Po_2, mm Hg
#
*
1
2
3
4
5
6
7
Blood Collection


C.
100
75
50
25
Po_2, mm Hg
#
*
1
2
3
4
5
6
7
Blood Collection